

CLAIMS

- Sub A1
1. Nucleotide or polynucleotide sequences deleted from the genome of *M. bovis* BCG/*M. bovis* and present in the genome of *M. tuberculosis* or conversely chosen from the following ORFs and genes: Rv2346c, Rv2347c, Rv2348c, *plcC*, *plcB*, *plcA*, Rv2352c, Rv2353c, Rv3425, Rv3426, Rv3427c, Rv3428c, Rv1964, Rv1965, *mce3*, Rv1967, Rv1968, Rv1969, *lprM*, Rv1971, Rv1972, Rv1973, Rv1974, Rv1975, Rv1976c, Rv1977, *ephA*, Rv3618, Rv3619c, Rv3620c, Rv3621c, Rv3622c, *lpqG*, *cobL*, Rv2073c, Rv2074, Rv2075, *echA1*, Rv0223c, RvD1-ORF1, RvD1-ORF2, Rv2024c, *plcD*, RvD2-ORF1, RvD2-ORF2, RvD2-ORF3, Rv1758.
2. The nucleotide or polynucleotide sequences as claimed in claim 1 grouped together in nucleotide regions RD5 to RD10 and RvD1 and RvD2 according to the following distribution:
- RD5: Rv2346c, Rv2347c, Rv2348c, *plcC*, *plcB*, *plcA*, Rv2352c, Rv2353c,
 - RD6: Rv3425, Rv3426, Rv3427c, Rv3428c,
 - RD7: Rv1964, Rv1965, *mce3*, Rv1967, Rv1968, Rv1969, *lprM*, Rv1971, Rv1972, Rv1973, Rv1974, Rv1975, Rv1976c, Rv1977,
 - RD8: *ephA*, Rv3618, Rv3619c, Rv3620c, Rv3621c, Rv3622c, *lpqG*,
 - RD9: *cobL*, Rv2073c, Rv2074, Rv2075c,
 - RD10: *echA1*, Rv0223c,
 - RvD1: RvD1-ORF1, RvD1-ORF2, Rv2024c
 - RvD2: *plcD*, RvD2-ORF1, RvD2-ORF2, RvD2-ORF3, Rv1758.
3. A method for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising the following steps:

- SubA1
- a) isolation of the DNA from the biological sample to be analyzed or production of a cDNA from the RNA of the biological sample,
 - b) detection of the DNA sequences of the mycobacterium present in said biological sample,
 - c) analysis of said sequences with the nucleotide and polynucleotide sequences as claimed in claim 1 or 2.

4. The method as claimed in claim 3, in which the detection of the mycobacterial DNA sequences is carried out using nucleotide sequences complementary to said DNA sequences.

5. The method as claimed in claim 3 or 4, in which the detection of the mycobacterial DNA sequences is carried out by amplification of these sequences using primers.

6. The method as claimed in claim 5, in which the primers have a nucleotide sequence chosen from the group comprising SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16, SEQ ID No. 17, and SEQ ID No. 18 with:

- the pair SEQ ID No. 3/SEQ ID No. 4 specific for RD5,
- the pair SEQ ID No. 5/SEQ ID No. 6 specific for RD6,
- the pair SEQ ID No. 7/SEQ ID No. 8 specific for RD7,
- the pair SEQ ID No. 9/SEQ ID No. 10 specific for RD8,
- the pair SEQ ID No. 11/SEQ ID No. 12 specific for RD9,

Sub A1

- the pair SEQ ID No. 13/SEQ ID No. 14 specific for RD10,
- the pair SEQ ID No. 15/SEQ ID No. 16 specific for RvD1, and
- 5 - the pair SEQ ID No. 17/SEQ ID No. 18 specific for RvD2,

7. The method as claimed in claim 6, in which the group from which the primers are chosen comprises, in addition, the nucleotide sequences SEQ No. 1 and SEQ No. 2, the pair SEQ ID No. 1/SEQ ID No. 2 being specific to RD4.

Sub A2

8. A method for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising the following steps:
- a) bringing the biological sample to be analyzed into contact with at least one pair of primers as defined in claim 6 or 7, the DNA contained in the sample having been, where appropriate, made accessible to the hybridization beforehand,
 - b) amplification of the DNA of the mycobacterium,
 - 25 c) visualization of the amplification of the DNA fragments.

9. A kit for the discriminatory detection and identification of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample comprising the following elements:
- a) at least one pair of primers as defined in claim 6 or 7,
 - b) the reagents necessary to carry out a DNA amplification reaction,
 - 35 c) optionally, the necessary components which make it possible to verify or compare the sequence and/or the size of the amplified fragment.

00936522 001401

Sub 12

- 5
10. The use of at least one pair of primers as defined in claim 6 or 7 for the amplification of a DNA sequence from *M. bovis* BCG/*M. bovis* or *M. tuberculosis*.
- 10
11. A product of expression of all or part of the nucleotide or polynucleotide sequences deleted from the genome of *M. bovis* BCG/*M. bovis* and present in *M. tuberculosis* or conversely as defined in claim 1.
- 15
12. A method for the discriminatory detection *in vitro* of antibodies directed against *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample, comprising the following steps:
- 20
- a) bringing the biological sample into contact with at least one product as defined in claim 11,
- b) detecting the antigen-antibody complex formed.
- 25
13. A method for the discriminatory detection of a vaccination with *M. bovis* BCG or an infection by *M. tuberculosis* in a mammal, comprising the following steps:
- 30
- a) preparation of a biological sample containing cells, more particularly cells of the immune system of said mammal and more particularly still T cells,
- b) incubation of the biological sample of step a) with at least one product as defined in claim 11,
- 35
- c) detection of a cellular reaction indicating prior sensitization of the mammal to said product, in particular cell proliferation and/or synthesis of proteins such as gamma-interferon.
14. A kit for the *in vitro* diagnosis of an *M. tuberculosis* infection in a mammal optionally

T04T60"E259E660

Sub A2
vaccinated beforehand with *M. bovis* BCG comprising:

- 5 a) a product as defined in claim 11,
b) where appropriate, the reagents for the constitution of the medium suitable for the immunological reaction,
c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction,
10 d) where appropriate, a reference biological sample (negative control) free of antibodies recognized by said product,
e) where appropriate, a reference biological sample (positive control) containing a
15 predetermined quantity of antibodies recognized by said product.
15. A mono- or polyclonal antibody, its chimeric fragments or antibodies, characterized in that
20 they are capable of specifically recognizing a product as defined in claim 11.
16. A method for the discriminatory detection of the presence of an antigen of *M. bovis* BCG/ *M. bovis*
25 or *M. tuberculosis* in a biological sample comprising the following steps:
a) bringing the biological sample into contact with an antibody as claimed in claim 15,
b) detecting the antigen-antibody complex formed.
- 30 17. A kit for the discriminatory detection of the presence of an antigen of *M. bovis* BCG/*M. bovis* or *M. tuberculosis* in a biological sample comprising the following steps:
35 a) an antibody as claimed in claim 15,
b) the reagents for constituting the medium suitable for the immunological reaction,

Sub A2
c) the reagents allowing the detection of the antigen-antibody complexes produced by the immunological reaction.

- 5 18. An immunological composition, characterized in that it comprises at least one product as defined in claim 11.
- 10 19. A vaccine, characterized in that it comprises at least one product as defined in claim 11 in combination with a pharmaceutically compatible vehicle and, where appropriate, one or more appropriate immunity adjuvants.
- 15 20. A method for the discriminatory detection and identification of *M. bovis* BCG or *M. tuberculosis* in a biological sample comprising the following steps:
- 20 - digestion with *HindIII*, of at least part of the genome of the mycobacterium present in a biological sample to be analyzed, and
- analysis of the restriction fragments thus obtained.
- 25 21. The method as claimed in claim 20, in which the analysis of the restriction fragments consists in counting said fragments and/or in determining their length.
- 30 22. Method of detection as claimed in either of claims 20 and 21, in which the analysis of the restriction fragments consists in bringing them into contact with at least one probe under stringent hybridization conditions and in
- 35 identifying the fragment parts or fragment hybridized.
23. A method as claimed in claim 22, characterized in that the probe is obtained by amplification of the

00036523-091401

Sub A2
genomic DNA with primers chosen from the group
SEQ ID No. 31, SEQ ID No. 32, SEQ ID No. 33 or
SEQ ID No. 34 with the pair:

- SEQ ID No. 31/SEQ ID No. 32 specific for DU1
- SEQ ID No. 33/SEQ ID No. 34 specific for DU2

24. Method according to claim 20, characterized in
that amplification is carried out of the fragments
obtained with primers chosen from the group
SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21,
SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24,
SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27 and
SEQ ID No. 28 with:

- SEQ ID No. 19, SEQ ID No. 20/SEQ ID No. 21
specific for JDU1
- SEQ ID No. 22, SEQ ID No. 24/SEQ ID No. 23,
SEQ ID No. 25 specific for JDU2A
- SEQ ID No. 26/SEQ ID No. 27, SEQ ID No. 28
specific for JDU2B

Sub A3
25. The method as claimed in claim 20, characterized
in that the fragments obtained are amplified with
primers chosen from the group SEQ ID No. 35,
SEQ ID No. 36, SEQ ID No. 37 and SEQ ID No. 38
specific for DU1 and then to analyze them by
sequencing.

Add
A4

Add
B1

Add
C2

0936523-091401